Scientific report

Concerning the deployment of the project in may-december 2013

During phase I of the project entitled "A new anti-invasive experimental strategy for infiltrative malignant gliomas", project code PN-II-RU-TE-2012-3-0235, the following objectives, proposed in the realization plan, were achieved:

1. The evaluation of the genes involved in invasion of gliomas in the glioblastoma samples and in the primary glioblastoma culture, through qPCR analysis.
2. Evaluation of the genes involved in glioma invasion in the glioblastoma lines through qPCR
3. Development of a new experimental glioblastoma invasion model: model type "organotypic brain slices"

In the development of these objectives, the following activities took place:

A. Harvesting the glioblastoma samples through classical surgical procedure, " open surgery" (Activ. 1.1) and stereotactic biopsy procedure (Activ. 3.1)
B. Obtaining the primary glioblastoma cultures and purchasing the glioblastoma lines (Activ.1.2 and Activ.2.1)
C. ARN extraction and deposit in a biological bank (Activ.1.3 and Activ.2.2)
D. qPCR analysis of the genes involved in glioblastoma invasion (Activ.1.4 and Activ.2.3)
E. Tissular sample extraction and processing, cultivation using the "organotypic brain slices" model and evaluation of the cell viability in the culture (Activ.3.2.)

A. Harvesting the tumor samples

Harvesting the glioblastoma samples used in this project has been accomplished through standard neurosurgical procedure ("open surgery ") or by stereotactic biopsy procedure. Tumoral fragments were obtained, extracted according to the standard surgical protocol, the tumor sample was selected from several fragments and directed towards microscopic analysis. Thus, tumor sample harvesting from this study did not influence the surgical or rezection grade, nor in the "open surgery", and it did not prolong the stereotactic biopsy procedure time. Sample harvesting was conducted in full safety for the patient and consent for given both by the patient and the patient's caregivers (Fig.1)

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Consimţământ informat pentru inrolarea in studiul:
„A new anti-invasive experimental strategy for infiltrative malignant gliomas"

Stimata doamna/Stimate domn,

Sunteţi invitat(ă) să participaţi într-un studiu despre modificările genetice în tumorile cerebrale. Scopul studiului nostru este de a identifica mecanismele moleculare implicate in invazia glioamelor cerebrale, pentru imbunatatirea diagnosticului si terapiei.
Participarea dumneavoastra este voluntara.

PROCEDURĂ: Veţi fi rugat(ă) să oferiţi o probă de tesut tumoral in timpul operatiei.
In timpul operatiei, o mica portiune din tesutul operat va fi prelevat in vederea extractiei de acizi nucleici si proteine pentru identificarea unor markeri specifici tumorilor cerebrale.
Dorim ca după încheierea studiului de faţă să păstrăm eventualul rest din proba ADN/ARN/proteica obţinută. Aceasta va fi congelată şi depozitată sub un cod şi nu direct cu numele dumneavoastră.
În cazul în care doriţi, vă puteţi retrage oricând din studiu, fără ca aceasta să afecteze în vreun fel dreptul dumneavoastră la tratament, inclusiv după ce aţi semnat acest formular.
Rezultatele studiului pe proba dumneavoastră sunt confidenţiale şi vor fi folosite numai în scop de cercetare.

BENEFICII ale participării:
- Ajuta la imbunatatirea protocolului de diagnostic
- Ajuta la dezvoltarea de noi terapii

RISCURI:
- Nu sunt riscuri suplimentare fata de cele asumate in consimtamantul operator

PLĂŢI ŞI ALTE BENEFICII:
- Nu veţi beneficia terapeutic din acest studiu, deoarece în acesta nu se administrează niciun medicament
- Nu veţi fi plătit(ă) ca să participaţi la acest studiu

CONFIDENŢIALITATE ŞI STATUT
Doar cercetătorii implicati in proiect şi un reprezentant al Comitetului de Etică al Spitalului vor avea acces la datele adunate pe parcursul acestui studiu. Utilizarea unor informaţii de tip personal este securizată conform legislaţiei în vigoare.
Deoarece informaţiile despre dumneavoastră şi despre starea dumneavoastră de sănătate sunt personale şi private, ele nu pot fi folosite în scop de cercetare fără acordul scris al dumneavoastră. Semnând acest formular, ne veţi da acordul dumneavoastră în acest sens.
Acest formular are scopul de a vă informa asupra felului în care datele despre sănătatea dumneavoastră vor fi folosite în acest studiu. Vă rugăm să citiţi cu atenţie înainte să semnaţi.

DATE DE CONTACT

Dacă aveţi întrebări legate de studiu sau dacă apar probleme, puteţi contacta persoana responsabila de studiu:

Dr. Felix Mircea Brehar, Spitalul Clinic de Urgenta „Bagdasar-Arseni", cu sediul în Bucuresti, cod poştal 041915, str. Soseaua Berceni nr. 10-12, judeţ (sector) 4, tel. 0213343025/int. 1707, mobil: 0724257549, fax 0213347350.

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Declaraţia pacientului

(Incercuiti raspunsul corect)

Sunt / nu sunt de acord ca probele mele biologice să fie folosite in cercetări ulterioare

Sunt / Nu sunt de acord cu testul/testele descrise în acest formular.

- Sunt de acord ca probele sa fi depozitate în Laboratorul de Cercetare al spitalului pentru uz ulterior.
- Înţeleg faptul că proba ar putea fi trimisă spre un alt laborator în afara Spitalului Clinic de Urgenta „Bagdasar-Arseni".

Semnătura pacientului .... Data.................................

Nume (Litere de tipar) ....CNP.................................

Semnătură apartinator ruda gradul I.. Dată ....

Nume (Litere de tipar) ....

Note importante: (bifaţi dacă este cazul)
- **Pacientul şi-a retras consimţământul (rugaţi pacientul să semneze aici) ....**
Data:.........................

Declaraţia personalului medical

Am explicat procedura pacientului. În mod particular i-am explicat beneficiile şi riscurile aşa cum apar în acest formular.
Am discutat de asemenea ce ar putea implica procedura, beneficiile şi riscurile oricărei testări alternative (inclusiv lipsa unei testări) şi orice problemă care preocupă pacientul.

Semnătura:.. Data ....

Nume (Litere de tipar) ....

Statut (investigator / medic curant) ....

Fig.1 – Consent form model, filled in by the patient and the guardian in the present study

Grade II,II,III cerebral glioma (glioblastoma) patients were included in the present study, diagnosis was confirmed by the hisotpathological examination (parafined samples colored with HE) ± imunohistochemical analysis. The reason why grade I cerebral gliomas were not included in this study is the fact that this type of tumors have distinctive histopathological particularities from the other glade gliomas (like the policytic astrocytoma) moreover, they are circumscribed and they do not have a tendency towards invasion (1,2).

a. Sample harvesting through classical neurosurgical "open surgery"

The neurosurgical technique of tumor removal was selected for good delimited tumors, localized in the ineloquent cerebral areas, accessible only through surgery and with an important mass effect on the adjacent cerebral structures (Fig.2), at which the initial plan was mass reduction at which we could anticipate a tumor resection as large as it could be without any major risk of postoperative neurological deficits.

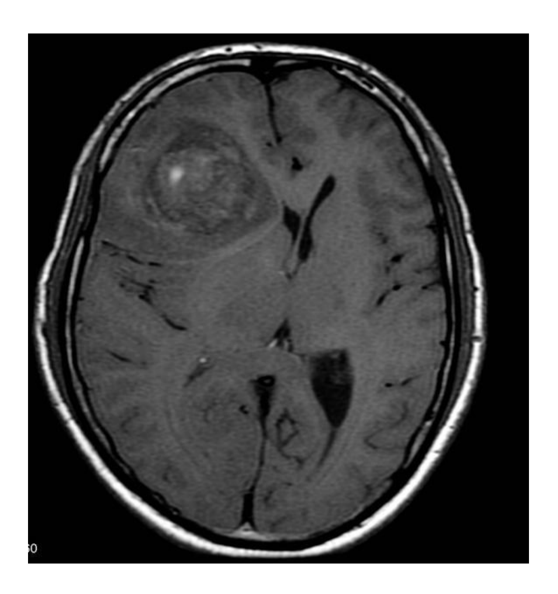


Fig.2. Right frontal glioblastoma (grade IV glioma). The tumor has a relatively good delimitations, localized in the right frontal hemisphere (non-dominant hemisphere) with mass effect. Patient has indications for “open surgery”.

Steps of the operations:

- Induction of anesthesia (general anesthesia with oro-tracheal intubation)
- Positioning and preparations of the operatory field
- Craniotomy
- Opening the dura mater and explosion of the tumor-infiltrated cerebral area that holds edema and modified vascular pattern
- Tumor removal
- Hemostasis
- Closing of the plans

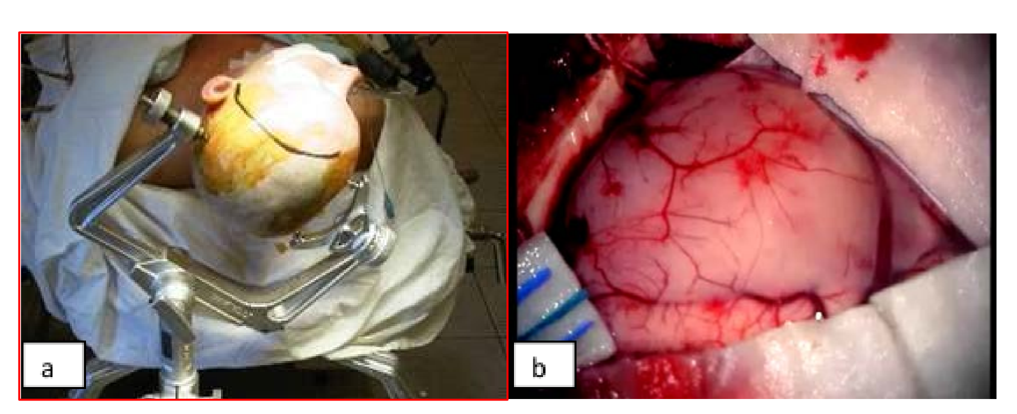


Fig. 3 Operatory steps of the neurosurgical intervention “open surgery”

B.Sample harvesting through stereotactic biopsy

Patients selected for stereotactic biopsy presented infiltrative cerebral gliomas (Fig. 4a), localized in profound or eloquent cerebral areas (Fig. 4b), at which tumor exertion could not be accomplished without a major risk of postop neurological deficits.

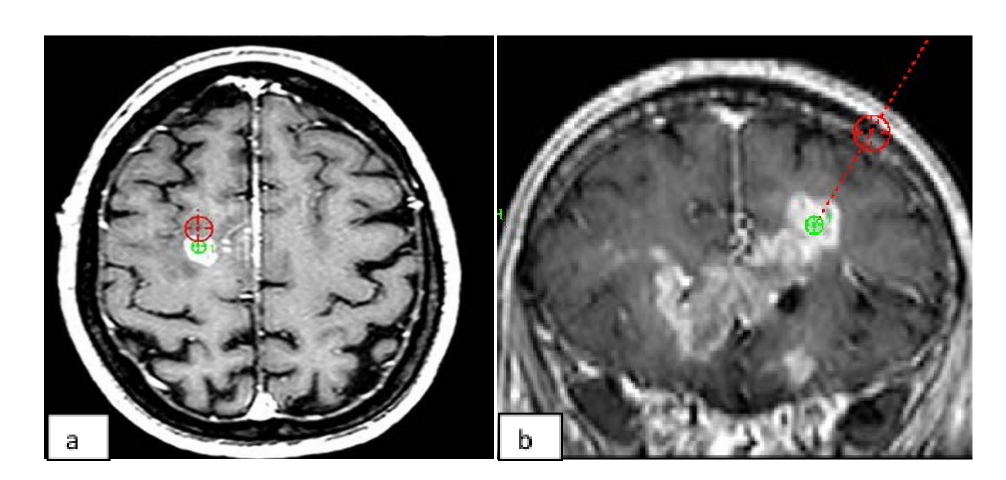


Fig.4 Types of cerebral gliomas selected for the stereotactic biopsy (T1 MRI with snapshot contrast, selected during the preoperator time) – Sp. CL. “Bagdasar-Arseni”

All stereotactic procedures were done by Dr. Felix Brehar, using the Leksell stereotactic system (Fig.5) and Stereotactic Planning System software (SPS), versiune NTPS 8.2 (Elekta, Sweden). For the scanning of the patient, type 1.5 MRI Tesla Magnetinc Resonance (Philips Integra). The system utilized by the author for the finalization of the procedure is one of the most error-free (medial error below 0.5mm and max below 1mm). The biopsy needle used was Type I Sedan (Elekta, Sweden) with a slot of 10mm. The steps of the stereotactic bypsy are :

-fixation of the stereotactic frame

-MRI or CT scan of the brain

-procedure planning

-stereotactic biopsy (fig.6)

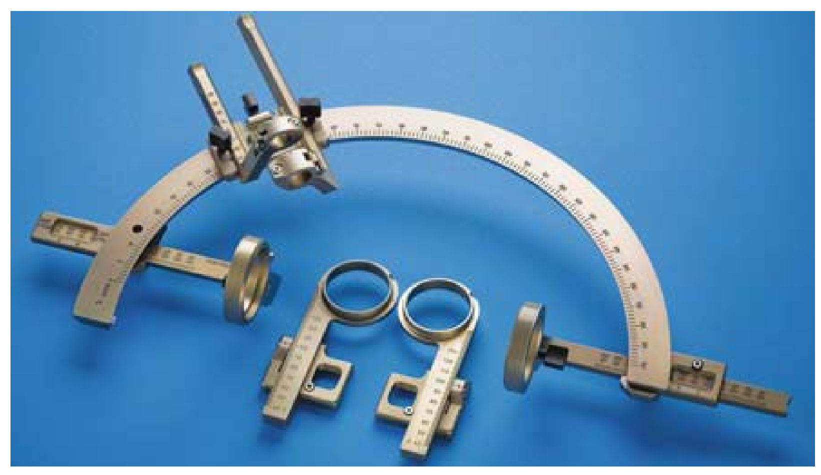

Fig. 5. Leksell stereotactic system



Fig. 6. Intraoperatory snapshot during the procedure of the stereotactic cerebral biopsy – Sp. Cl. “Bagdasar-Arseni”

Intraoperative tumor samples selected for inclusion in the study had sizes less than 1 cm, were cleaned of blood and cellular debris and were included in a sterile conditions in eppendorf tube filled with 1.5 ml RNA solution saver and were immediately stored at 2-4 ° C 24 hours and then at -80 ° until RNA extraction.

By the time of reporting 30 patients were included in the study with brain gliomas in which from 15 patients tumor samples were collected by standard surgical technique "open surgery" and 15 were performed the stereotactic biopsy. In 11 patients in which the tumor was located in ineloquent areas in the frontal and temporal pole, standard resection procedure was performed for frontal and temporal pole. In these cases we have achieved complete tumor ablation and harvesting of the peritumoral cerebral tissue could be performed in safety conditions. These samples have been utilized as a reference. We have extracted RNA from 21 out of 30 cases, having a total of 31 probes by the time of the reporting (21 tumor and 10 peritumoral).

b.Obtaining the primary glioblastoma cultures and purchasing of the glioblastoma lines

In two cases with high-volume tumors (patient 15 and 16) at which the open neurosurgical intervention was performed, sampling of several femoral fragments was possible, from which primary glioblastoma cultures were initiated. It is very important to mention the time the fragments stayed in physiological serum or culture environment until processing. Processing of the tumoral fragments was performed in the same day, at a maximum of 2-3 hours from intraoperative harvesting. It the preservation time of the tumors surpasses several hours, it is expected that enzymatic extra and intracellular reactions should be induced, with cellular damage, modification of the tumor cells properties, viability decrease through membrane sensibilization at the action of enzymatic systems, the lowering of the adhesion rate post cryoconservation, until massive cellular destruction through osmotic lysis, enzymatic etc.

The processing of the fragments is done in perfect sterile conditions, at a laboratory hood.

Before mechanic processing, the tumor fragments were washed 3 times in physiological serum or PBS (phosphate buffer solution), for the removal of blood stains and cellular debris.

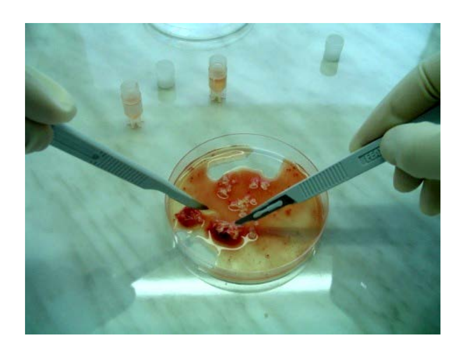


Fig. 7. Mechanical processing of the tumoral fragment in the laboratory hood

Processing of the eloquent cellular groups is done with soft instruments, extremely sharp, sterile, microsurgical instrumentary, in a Petri dish of medium dimensions. It is extremely important in microscopical recognition of the relevant tumoral parts, removal using a scalpel blade of the necrosis, coagulated portions, cerebral veins/arteries or neoformation tumor cells and their clots, fibrous zones from the tumor capsule, gliosis cerebral portions, normal peritumoral brain-tissue, fat tissue fragments, muscular, etc. The relevant tumor parts are of brown-grey-red color, and their recognition is possible only through gained experience, from multiple tumor exertions. After eliminating the irrelevant tumor parts, the viable tumor remainder is sectioned repeatedly with a scalpel, until milimetrical fragments ar obtained.

The steps of primary glioblastoma cultivations were :

1. Enzymatic dispersion and mechanical tissular fragmentation
2. Suspension in culture environment DMEM with 20% fetal serum
3. Seriate confluence subcultures 85-90%

The utilized environments were : DMEM (Dulbecco Modified Essential Medium) +3% Penicillin and Streptomycin +20% fetal serum, PBS(Phosphate buffered saline solution) 0,01M, Tripsine 1:250, 1% glucose.

Tissular dispersion proved to be more efficient and much faster when tripsine solution was used compared to EDTA, in exchange, cellular adhesion and monolayer formation occurred much slower with enzymatic dispersion. As a result, fragment dispersion through tripsine and EDTA, although slower, it protects cells and favorize adhesion and etalation.

The dispersion time is maintained at 2-3 minutes; the membrane integrity is damaged at over 5-8 minutes and the cells do not adhere. EDTA in optimum concentration 20mM act as a chelating agent of Ca+2 (Ca+2 is active in the intercellular adhesion). 1% Glucose of tripsine assures a lager percentage of viable cells if adequate osmolarity.

Also, experimentally we noticed that tripsine inactivation is more efficient if it is achieved by adding fetal serum as opposed to ice inactivation.

We have cultivated cells for 20 passages, observing the fenotipic aspect.

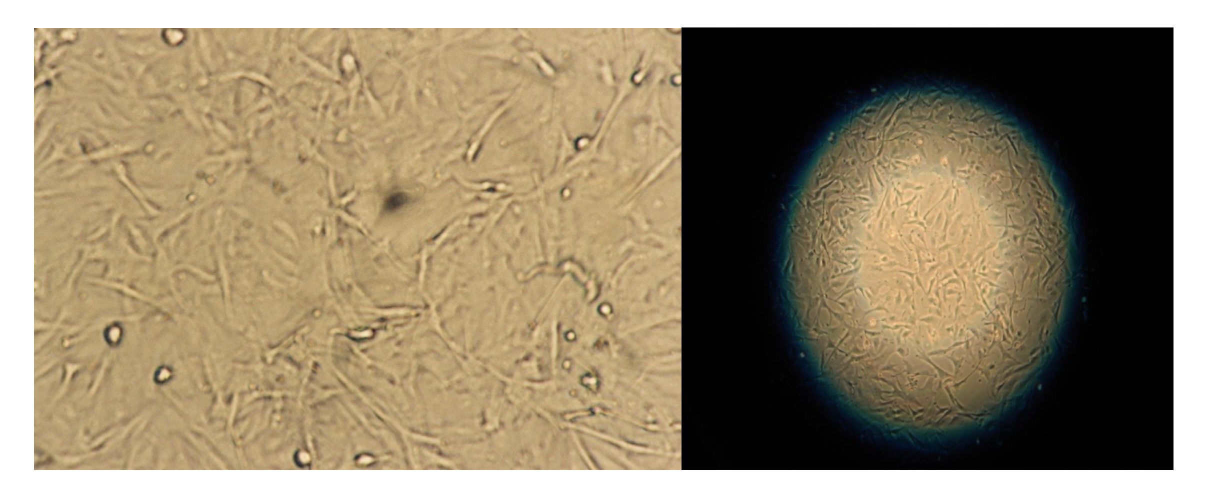


Fig. 8. Microscopic view (microscop Zeiss Axiovert 25C) of primary glioblastoma culture at passage (left) and 20 respectively (right).

During this project, we have purchased glioblastoma lines U-251 MG (initially named U-373 MG) from the European Collection of Cell Cultures (ECACC). This is one of the oldest and used cell-line of glioblastoma and it is very useful in this project because it assures a high occurrence of the events resulted from the experiments(4). This line was delivered in frozen criocilinders. For the revitalization we have used the usual revitalization process, and we have used the following compounds:

minimum essential medium(MEM), nonessential amino acid solution, piruvic solution, fetal serum, antibiotic solution, glutamine solution. U251 cell-line is passed at a confluence of 60-70%. The phenotypic aspect is shown is Fig.9

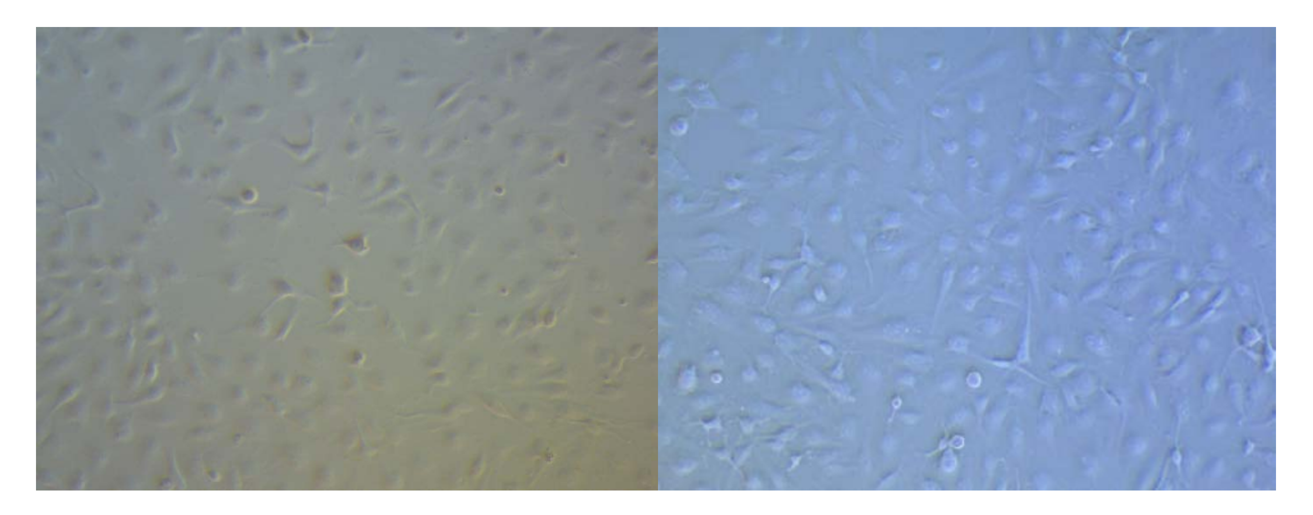


Fig.9. Microscopic aspect of U251 glioblastoma culture.

C. ARN extraction and biobank deposit

The samples were harvested from the patients, washed in PBS and stored in RNASave. The total ARN was isolated with the use of the Maxwell KIT 16 LEV simply RNA and Maxwell apparatus (Promega). In order to distinguish the RNA, the samples were mechanically fragmented and homogenized in tampon from the Maxwell 16 LEV simply RNA kit, with the help of a zirconium ball of 0,5mm diameter (circa 200mg). Homogenization was done in 2 cycles of 30sec each, separated by 1 30sec cooling cycle, using the Speed Mill (Analytik-Jena, Germany) apparatus. After centrifuge of the samples 1 min 5000g, 200uL of supernatant was mixed with 200uL of lysis buffer, strongly vortexed 15sec and inserted in the cartridge of the Maxwell 16 apparatus, where RNA isolation took place. The concentration of the RNA samples was evaluated by reading the optical density at 260nm at the Nanodrop spectrophotometer.

The isolated RNA quality was evaluated by determining the D0260/D0280 coefficient. All the samples had coefficients from 1.8-2, which indicates a high RNA purity isolated from the tissue. RNA with a weight ranging from 0.800 ug to 37 ug per probe was obtained. For reverse transcription of the qPCR we have used 500ng of RNA for each probe. RNA excess was deposited in a -80 degrees C biobank for further experiments.

D. qPCR analysis of genes involved in glioma invasion

In this project we have analyzed the expression of the following genes in the tumor tissue: PAFAH1B1(LIS1), NDEL1,CDK5, MYH9,TWIST1,SNAI2. LIS1, NDEL1 and CDK5 are components of the pro-neural pathway (molecular mechanism similar with the one utilized by the precursor neural bipolar migrating cells, during cerebral ontogenesis)(5,6), time in which TWIST1 and SNAI2 genes are part of the pro-mesenchymal components (molecular mechanism utilized by other types of tumor during metastasis)(7,8). MYH9 (myosin II) is a molecular engine proved to be involved in the migration of glial

tumor cells(5,6). As a reference expression we have used two housekeeping genes Actin B (ACTB) and GAPDH.

Reverse transcription RNA in cDNA were achieved using MMLV and oligo (invitrogen) and 500ng RNA, in a final volume of 50uL.

Real time PCR was achieved using the TaqMan Gene Expression Assays (Invitrogen) for the following genes:

- PAFAH1B1 (Assay ID: Hs00181182_m1),

- CDK5(Assay ID: Hs00358991_g1)

- MYH9 (Assay ID: Hs00159522_m1)

- TWIST1 (Assay ID: Hs01675818_s1

- SNAI2 (Assay ID: Hs00950344_m1)

- NDEL1 (Assay ID: Hs01092624_m1)

All probes for the aforementioned genes have been marked with FAM.

For the normalization of the results, we have analyzed the expression of the GAPDH and actin genes, and the probes belonging to them have been marked VIC.

The reaction mixture contained 1 uL of cDNA, 5uL TaqMan Universal Master Mix II, with UNG (concentrated x2m Invitrogen), 1uL primer and probes and 3ul water. Pipetting the probes on 384 wells clips has been achieved with the use of Qiagility(Qiagen) automated pipettor, using conductive 50uL tips.

The amplification programme was the following: 2 min,50 degrees C;10min,95 degrees C;followed by 40 cycles : 15 sec, 95 degrees and 1 min, 60 degrees was achieved in the 7900HT system apparatus from Applied Biosystem.

The results obtained in the SDS2.4 programme, were processed using the analysis RO Manager software.

The Values of the genes expression traced in the tumor and peritumoral(normal) samples are shown in table 1.

proba	nr. pac.	Assay	CDK5	MYH9	NDEL1	PAFAH1B1	SNAI2	TWIST1	ACTB vic	GAPDH vic
normal	1	1 (RQ)	0,9714	4,9686	1,867	2,251	1,2173	9,9229	1,4379	0,6955

normal	3	4 (RQ)	0,9471	1,9536	0,9278	1,5451	1,1274	0,1206	0,8542	1,1707
normal	4	6 (RQ)	1,2088	0,163	0,8194	1,5359	1,6445	0,351	1,2206	0,8193
normal	7	10 (RQ)	1	1	1	1	1	1	1	1
normal	8	12 (RQ)	0,6236	3,2145	3,475	3,0225	1,5478	0,3872	1,1074	0,903
normal	10	15 (RQ)	1,4261	2,0422	0,636	1,0868	2,5491	156,1858	0,9353	1,0692
normal	13	19 (RQ)	4,25	3,5051	2,1324	3,3684	0,7871	12,4159	0,9345	1,0701
normal	19	26 (RQ)	3,2193	3,1348	1,5566	1,8181	1,4803	1,7799	0,9797	1,0207
normal	20	28 (RQ)	0,822	1,983	1,0389	0,5797	1,3632	1061,1481	0,5873	1,7027
normal	21	30 (RQ)	0,8343	0,872	0,9905	1,2614	0,6833	1,0912	0,7298	1,3703
tumora	1	2 (RQ)	0,593	2,2895	0,3814	0,1078	2,8505	370,6255	0,4495	2,2246
tumora	3	5 (RQ)	1,3599	0,6167	0,3603	0,6792	1,3771	145,1248	0,6365	1,5711
tumora	4	7 (RQ)	2,2943	1,8173	0,9126	0,9301	3,2428	5,2325	1,1277	0,8868
tumora	7	11 (RQ)	1,9776	3,397	0,5937	0,5791	2,7548	0,067	0,5612	1,7819
tumora	8	13 (RQ)	1,6677	4,168	0,9616	0,8178	0,4639	24,3219	0,8966	1,1153
tumora	10	16 (RQ)	0,2629	3,7631	1,083	2,4531	6,457	246,7301	1,0814	0,9248
tumora	13	20 (RQ)	0,7655	1,022	0,9997	0,7724	1,3143	6,6413	1,1229	0,8905
tumora	19	27 (RQ)	1,2173	0,7826	0,4704	0,9973	3,3011	34,1066	0,4203	2,3793
tumora	20	29 (RQ)	0,9382	2,2798	1,0087	0,8262	1,5112	1107,3166	0,7897	1,2663
tumora	21	31 (RQ)	0,7491	3,8306	0,696	0,6982	2,5612	41,4521	0,8546	1,1701
tumora	9	14 (RQ)	0,8238	1,2496	1,0989	0,5959	3,0797	0,2886	0,573	1,7452
tumora	24	17 (RQ)	1,5311	0,7033	0,7352	0,566	3,5391	463,5058	0,509	1,9647
tumora	25	18 (RQ)	1,843	1,295	0,8638	0,8936	2,8642	14,3021	0,5292	1,8896
tumora	14	21 (RQ)	1,1479	1,3218	0,4402	0,7901		0,1735	0,5098	1,9617
tumora	15	22 (RQ)	0,6088	0,4047	0,4127	0,2448	2,6895	0,0735	0,4612	2,1683
tumora	16	23 (RQ)	1,7441	0,6171	0,7332	1,9885	3,5162	106,554	0,5555	1,8001
tumora	17	24 (RQ)	2,5625	0,7935	1,34	2,2342	0,7049	0,1133	0,4568	2,1894
tumora	18	25 (RQ)	1,6222	1,5868	0,8481	1,1109	0,8291	59,7247	0,6923	1,4444
tumor	2	3 (RQ)	1,871	1,9149	1,0235	0,6593	0,588	0,1668	0,6758	1,4797

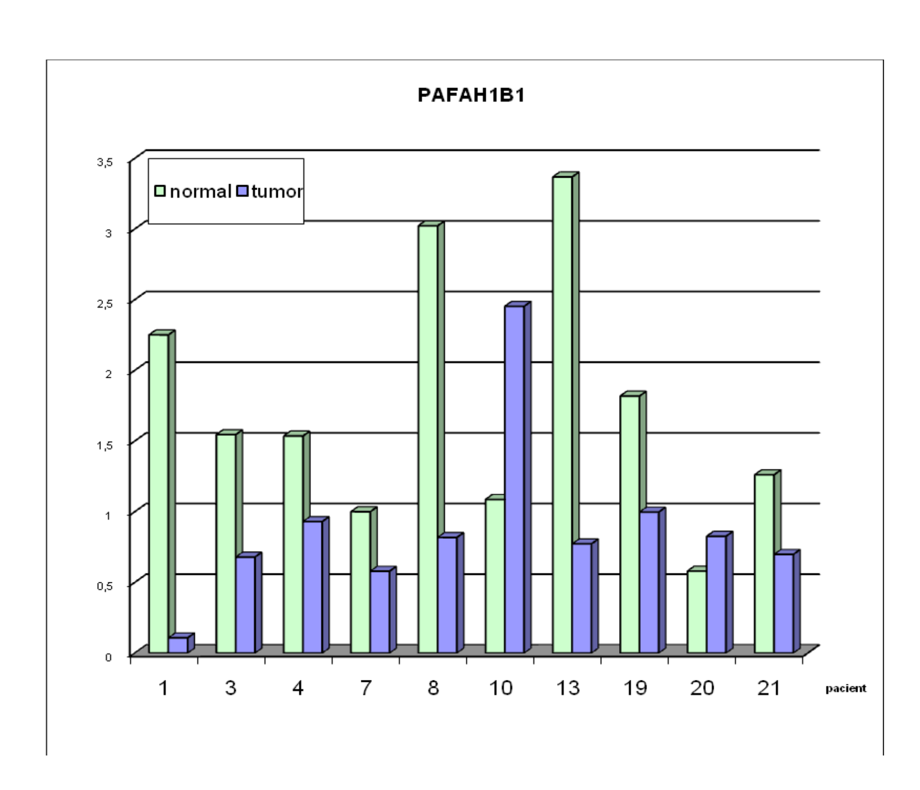
a			9				4			
tumora	5	8 (RQ)	4,0557	0,8265	4,1002	4,1463	7,2411	11,1016	3,1359	0,3189
tumora	6	9 (RQ)	0,1424	1,4381	0,5613	0,1722	1,2184	605,4901	0,5438	1,8388

Tabel 1. Gene expression in tumoral and normal samples

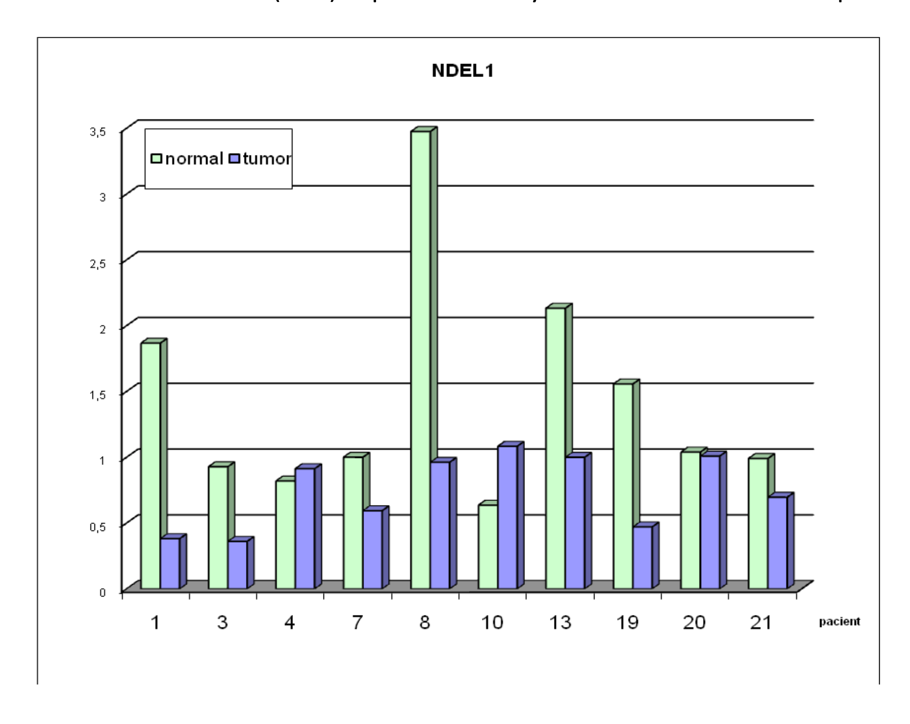
From the analysis from table 1 we can notice high values in certain samples tumoral and peritumoral (normal) for the TWIST gene, fact which makes it difficult to interpret the result obtained for this gene. Comparing the gene expression between tumoral and normal samples for the 10 patients from which we obtained both types is illustrated in table 2.

Nr. pacient	**CDK5**	**MYH9**	**NDEL1**	**PAFAH1B1**	**SNAI2**
1	**0,61**	**0,46**	**0,20**	**0,05**	**2,34**
3	**1,44**	**0,32**	**0,39**	**0,44**	**1,22**
4	**1,90**	**11,15**	**1,11**	**0,61**	**1,97**
7	**1,98**	**3,40**	**0,59**	**0,58**	**2,75**
8	**2,67**	**1,30**	**0,28**	**0,27**	**0,30**
10	**0,18**	**1,84**	**1,70**	**2,26**	**2,53**
13	**0,18**	**0,29**	**0,47**	**0,23**	**1,67**
19	**0,38**	**0,25**	**0,30**	**0,55**	**2,23**
20	**1,14**	**1,15**	**0,97**	**1,43**	**1,11**
21	**0,90**	**4,39**	**0,70**	**0,55**	**3,75**

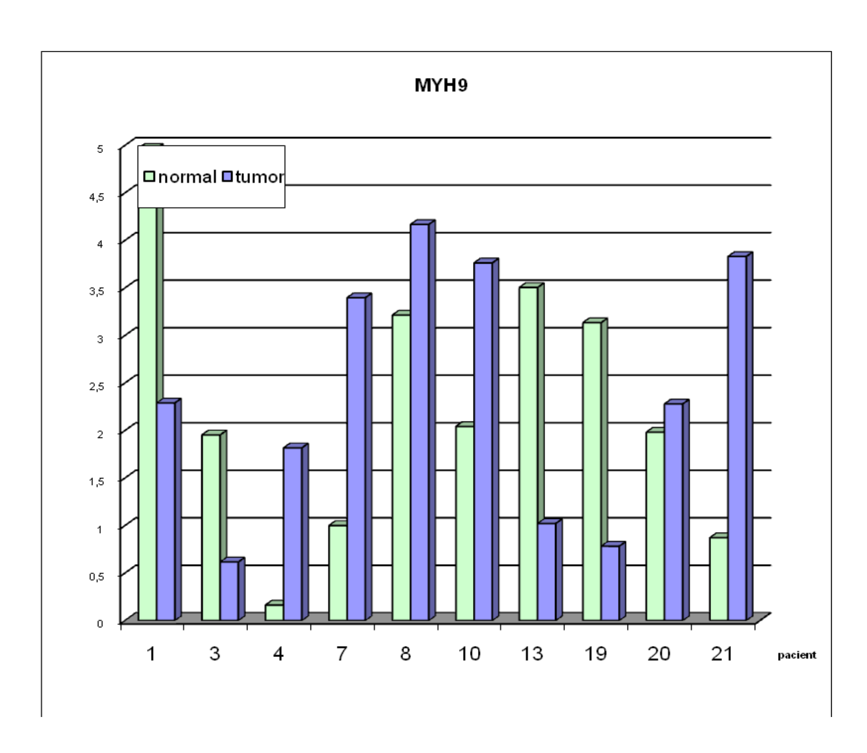
The graphic representation of the target genes in the tumoral tissue compared to the peritumoral for the 10 patients is shown in the following graphic.



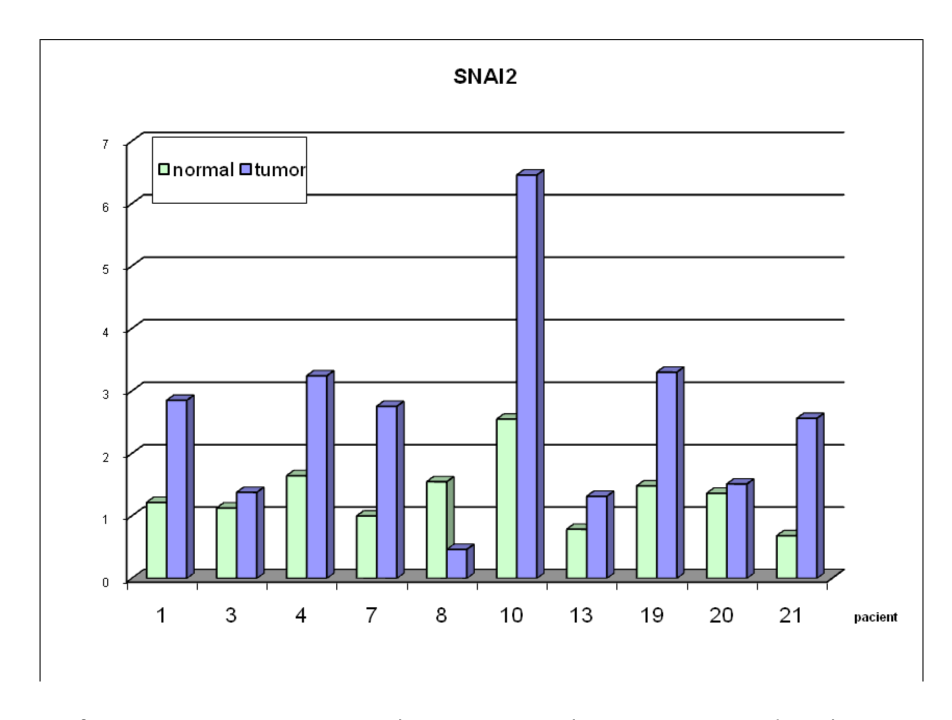
Grafic 1. PAFAH1B1 (LIS1) expression analysis in tumoral tissue compared to the peritumoral tissue



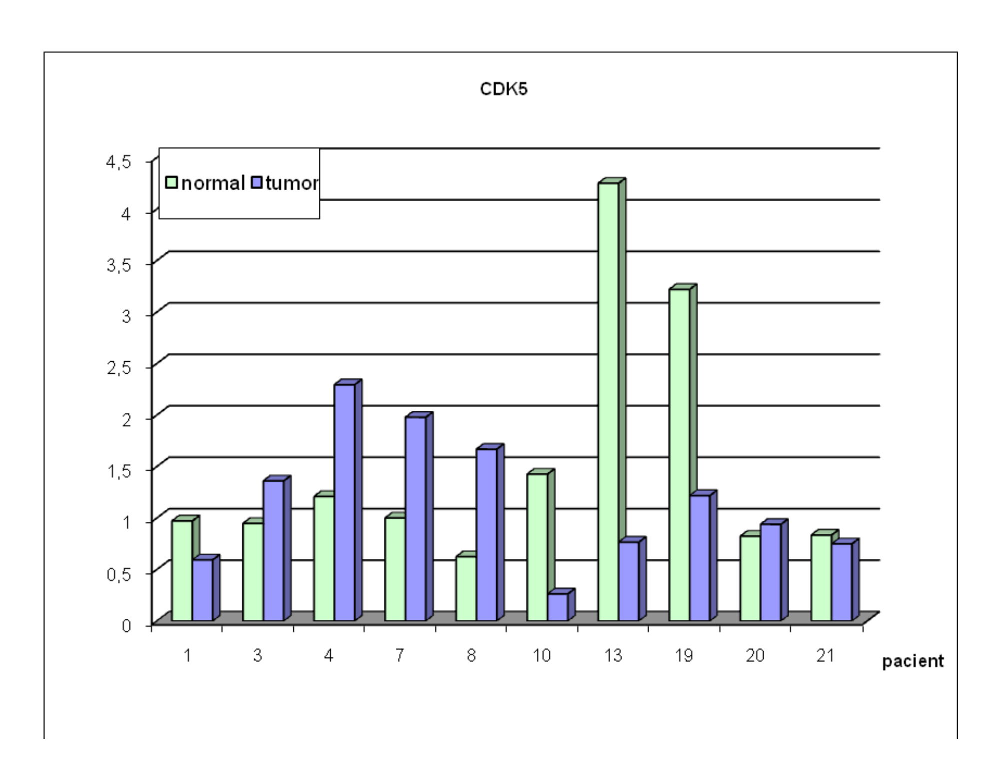
Grafic 1. NDEL expression analysis in tumoral tissue compared to the peritumoral tissue



Grafic 3. MYH9 expression analysis in tumoral tissue compared to the peritumoral tissue



Grafic 4. SNAI2 expression analysis in tumoral tissue compared to the peritumoral tissue



Grafic 5. CDK5 expression analysis in tumoral tissue compared to the peritumoral tissue

From the analysis of the target genes in the 10 cases at which we could harvest tumor samples as well as peritumoral (normal) samples, we have state that the SNAI2 gene that presents a constantly high activity in all tumoral samples except in case nr,8 (graphic 4). MYH9 gene present a high activity in 6 out of the 10 cases, CDK5 genes in 5 out of 10 cases and LIS and NDEL1 in 2 out of 10 cases.

Comparing the average expression of the target genes (except TWIST gene) in the tumoral tissue (21 samples) and peritumoral cerebral (10 samples) we have obtained the following results (table 3). We can observe a higher expression of the SNAI2 in tumor samples (with 95%) comparing the normal samples. SNAI2 gene is involved in the oncogenetic mechanism, specially in the invasion and metastasis of the carcinomas and we also proved the its high expression in malignant gliomas (10) as well as the correlation of the TWIST expression.SNAI2 gene may present as a molecular target in the anti invasive therapy and will be studied in the following steps of the project. For the TWIST gene we shall repeat the qPCR analysis.

Tabel 3: Average gene expression of genes in normal and tumoral samples

Medie	CDK5	MYH9	NDEL1	PAFAH1B1 (LIS1)	SNAI2
N	1,52	2,28	1,44	1,74	1,33
T	1,42	1,71	0,93	1,05	**2,60**

The expression of the genes involved in the pro-neural path is elevated in the cerebral tissue compared to the tumoral tissue. For the CDK5 gene this result was emphasized by other authors which noticed the CKD5 level in glioblastoma is lower (at a small difference, statistically insignificant) than the cerebral tissue, but is larger compared to the astrocit cells. This is due to fact that CDK5 levels is usually higher in neurons compared to astrocytes, thus knowing the fact that astrocit cells are the origin for grade II-IV gliomas, we can conclude that CDK5 is overstated in glial tumor tissue. This mechanism could explain other results achieved for the other genes involved in the pro-neural pathway (LIS1 and NDEL1) in which we have elevated expression in the cerebral tissue compared to the tumoral tissue.

E. Processing the extracted tissue samples, cultivated after the "organotypic brain slices" model and evaluation of the viability after the culture

One of the experimental models frequently used for the study of invasive gliomas is the in tissue model (Organotypic Brain Slice Culture) of cultivation for cerebral mouse tissue sections. This model does not reflect sufficiently enough the in situ biological reality. Thus there are differences between cellular morphology and characteristics of the mouse compared to the human and, moreover, at the periphery of the tumor occur some phenomenons such as : peritumoral edema, peritumoral gliosis, which influence the migration of tumoral cells. So a new element of the project is the development of a new in tissue model in glioblastoma invasion. For this purpose we have harvested using stereotactic tisular fragment that include the tumoral as well as the peritumoral portion which represents the transition area between normal cerebral tissue and tumor which is very important from a glioma invasion point of view. The initial target was to localize at the periphery of the hypersignal T1 contrast MRI area, for grade III and IV gliomas, as well as the periphery area of the hypersignal in the FLAIR sequence for grade II gliomas. Therefore the needle used for the biopsy was Sedan with a 10mm hole, on the same tissue sample we could identify at the histopathological analysis the tumoral tissue as well as the peritumoral cerebral tissue (Fig.9).

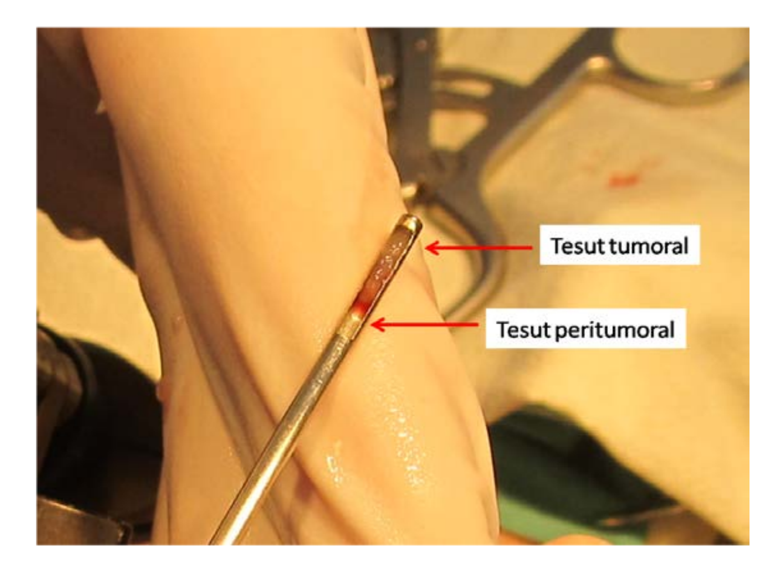


Fig.9. Intraoperative view of tissues samples obtained by stereotactic biopsy.

Tissular Cylinder was sectioned using the Mcllwain Tissue Chopper microtome in tissular section of approx 300-400 microns in depth. The sections were cultivated on specially treated culture clips (flat culture clips with 0.4 microns pores and a 12 and 30mm diameter), using the DMEM environment supplemented with glutamine fetal serum and AB solution at a temperature of 37 degrees C and CO_2 5%. On a period of 14 days we have observed the viability of tissular sections, with the preservation of the cellular architecture. The study of tumor-cell migration using tissue sections extracted from the tumor periphery and cultivated in vitro will be achieved in the following step of the project.

Results

The partial results of the project contributed at the achievement and presentation of the following papers at national and international congresses:

1. Nestin expression in biopsy samples correlates with the invasive phenotype of cerebral gliomas. F. M. Brehar, D. Arsene, M. Lisievici, M. R. Gorgan. Prezentare orala. 9th CONGRESS of the RSN with International Participation, 19-21 Septembrie, 2013, Bucuresti, Romania.

2. Glioma stem cells specifically induce infiltrative growth pattern xenografts. F. M. Brehar, R.M. Gorgan, C. Bleotu, O. Zarnescu. Prezentare poster. EANS Annual Meeting 2013, 11-14 Noiembrie 2013, Tel Aviv, Israel.

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